ADÉLIE PENGUIN (*PYGOSCELIS ADELIAE*) EMBRYONIC DEVELOPMENT AT DIFFERENT TEMPERATURES

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ABSTRACT.—Adélie Penguin eggs were artificially incubated at five different constant temperatures: 26°, 30°, 34°, 40°, and 42°C. A general increase in embryonic development rate was observed with higher incubation temperatures. Embryonic growth and development were not completely prevented at either 26° or 42°C. Mortality of embryos was lowest at 34°C, and embryonic death occurred more frequently during the first week of incubation at higher temperatures than at lower temperatures. Abnormal development was most common at temperatures other than 34°C. Some abnormal conditions were characteristic of low incubation temperatures while others were found most often at high temperatures. We suggest that the optimum constant incubation temperature for the Adélie Penguin embryo may be slightly higher than 34°C. *Received 9 June 1976, accepted 27 December 1977.*

DESPITE scientific study since the early Antarctic explorations, two aspects of the life history and adaptations of the unique Adélie Penguin (*Pygoscelis adeliae*) to its adverse environment are still not fully understood. Here we review current knowledge of the conditions necessary for optimum egg incubation, and present the results of our studies on the embryonic results of non-optimum incubation.

Adélie Penguin behavior studies by Sladen (1958), Penney (1968), and others suggest that the ability to incubate eggs successfully varies among individual birds. Yeates (1968) suggested that weather, especially Antarctic blizzards, may have a direct influence upon the length of time required to hatch eggs. That incubation temperatures may not be the same in all nests is suggested by means of 33.3, 34, and 37.9 days, and ranges of 30–37, 33–36, and 33–42 days, reported by Taylor (1962), Sladen (1958), and Yeates (1968), respectively, for the incubation period of this species. This variation is probably due to the combined effects of weather and an individual penguin's ability to incubate its eggs.

More direct evidence of incubation temperature variability, even at one nest, is given by Eklund and Charlton (1959), who found by telemetry that incubated egg temperature averaged 33.7°C, with a 29.2°–36.8°C range over a few days. Derksen (1977) recorded an average temperature of 35.2°C with a 30°–38°C range for an egg incubated by one bird for 27 h. All penguins do not incubate their eggs at the same temperature, and the incubation temperature under one bird is not constant.

Embryological studies of penguins, such as those of Waterston and Geddes (1909), Anthony and Gain (1915), Parsons (1932, 1934), Glenister (1954), O'Gorman (1964), and Herbert (1967, 1969), have essentially been confined to comparisons with the embryology of better known species such as the chicken (*Gallus domesticus*). Herbert (1967) summarized these earlier embryological studies and prepared a timed series of Adélie Penguin normal developmental stages.

Our primary objective was to determine the embryological effects of incubating Adélie Penguin eggs at sub- and supra-optimal temperatures. Herbert's (1967) timed series provided the basis for this determination. A secondary objective was to determine the incubation temperatures that limit embryonic growth and development. We also attempted to determine if the incubation temperature (33.5°–34.0°C) em-

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ployed by Herbert (1967) for his timed series is the optimal temperature for Adélie embryonic development.

METHODS

The 241 Adélie Penguin eggs for this study were collected in 1969 and 1970 from the Cape Hallett Rookery (72°19'S, 170°13'E). Hallett Station's proximity to this large number of nesting penguins facilitated egg collection without excessive cooling during transport to the incubators.

More than 200 nests in six colonies were marked before the appearance of the first egg. To minimize the effects of egg collection only first-laid eggs were removed. If these are taken soon after laying, a third egg is often laid to complete the normal clutch of two (Taylor 1962), as occurred in 54% of the nests we used.

Eggs destined for artificial incubation were collected within 3 h of laying and carried to the laboratory in insulated containers to reduce cooling. Nevertheless, the eggs were usually cool to the touch when they arrived in the laboratory. In 1969, due to a shortage of incubator space, 49 eggs were stored at 15° ± 2°C for 5–6 days before being placed in incubators. Eggs were placed in three incubators at different temperatures. An attempt was made to minimize the number of temporary temperature drops caused by egg addition or removal. In such cases the constant incubation temperature was regained within 30 min, and the change in egg temperature was probably insignificant. Humidity was provided by placing pans of water on the floor of each incubator and was supplemented by spraying the eggs with water twice per day. Eggs were turned at least once but usually twice per day.

Egg contents were removed and immersed in Ringer's chick-saline solution for embryo measurement and initial observation. The embryo was removed from the yolk, fixed in Bouin's solution, and stored in individual vials containing a 20% solution (by volume) of glycerol in 70% ethanol for transport to Iowa State University, where 206 embryos were stained with paracarmine and mounted whole on glass slides. Whole-mount embryos were microscopically examined, and developmental stages were determined according to Herbert's (1967) scheme. Measurements and sketches of these embryos from the preliminary examination complemented the stage determination from whole-mounts. Presence of heartbeat in large embryos proved their viability when first examined. Presence of mitotic figures and absence of necrotic cells were used to determine viability of embryos in which the heart was not yet formed.

RESULTS

Storage of eggs at 15°C had no consistent or significant effect upon embryo development rate, abnormality, or mortality when compared with embryos from unstored eggs that had been incubated for equal periods at the same temperature. Therefore we combined stored and unstored eggs in our analyses.

To provide a control, and for comparison with Herbert's (1967) timed developmental series, 40 eggs were incubated at 34° ± 1°C for various periods of time. Embryos from 28 of these were staged, and mean observed developmental stages were plotted against incubation period. Figure 1 compares these data with those reported by Herbert (1967). Although ranges are indicated differently for each curve the developmental rates they describe are comparable. Slightly faster development is apparent for eggs incubated at 34° ± 1°C (present study). The developmental stages (Fig. 1) correspond to those of Herbert (1967), and he chose his stage numbers to approximately correspond to those of Hamburger and Hamilton (1951). Stage 11, for example, resembles the "33-hour" chick embryo studied in embryology courses. Stage 18 (10 days of incubation) resembles the "72-hour" chick embryo that has a bent body, large head, and limb rudiments.

The rate of development increased with higher incubation temperatures from 26° to 42°C (Fig. 2). Incubation at temperatures other than 34°C, however, resulted in either increased mortality, increased abnormal development, or both (Table 1). Observed mortality was probably somewhat less than actual mortality because the samples indicated in Table 1 include some embryos that were not mounted, and it

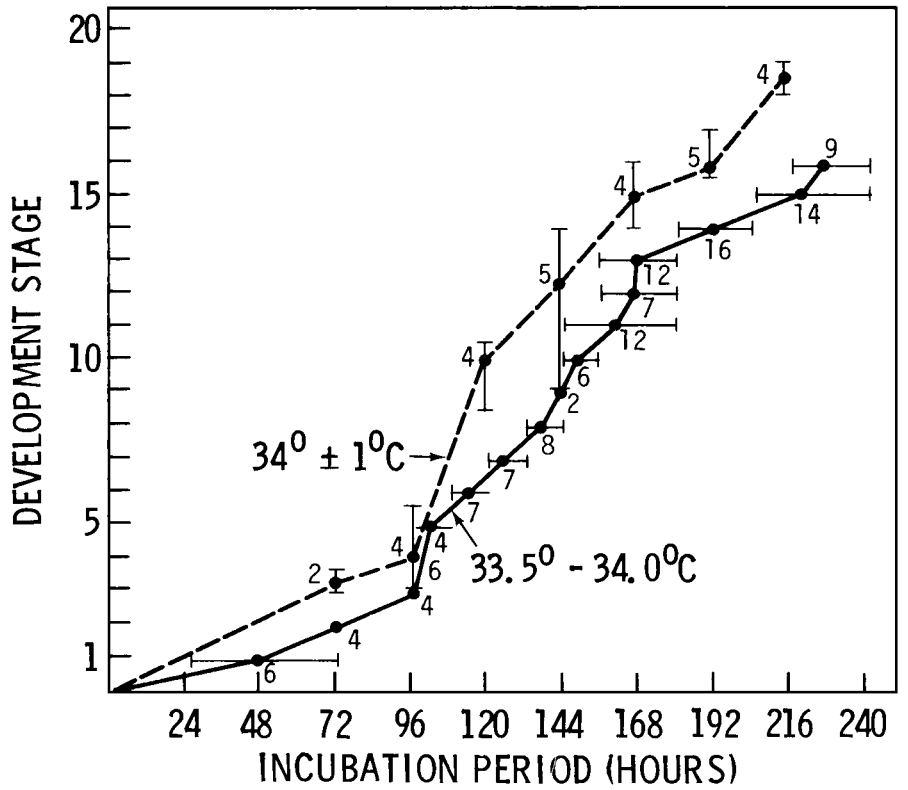

Fig. 1. Comparison of rate of development for eggs incubated at 33.5°–34.0°C (Herbert 1967) (solid line) and at 34° ± 1C (present study) (broken line). Numbers by points indicate sample size for the mean values. Range for each value is indicated by a horizontal line (h) for Herbert's (1967) data and a vertical line (stage) for data of the present study.

is likely that actual mortality was not determined for some of these unmounted specimens. Also, death of small embryos before fixation is difficult to detect, even with preserved specimens. Similarly, the occurrence of abnormal development was probably greater than that observed. Some abnormal development in unmounted and in dead embryos was probably not detected. In addition, only a few indications of abnormal development could be observed in small embryos, in which it was often difficult to distinguish normal from abnormal development.

The type of abnormal development observed was related to incubation temperature. The area pellucida and interior portion of the area opaca had degenerated leaving only a white ring of embryonic tissue in 25.0% of the embryos incubated at 40°C, in less than 1% of the embryos incubated at 30°C, and in none of those incubated at 34°C. Abnormal development of the brain region was observed in 17.0% of the embryos at 40°C, in 7.5% of the embryos at 34°C, and in only 1.4% of the embryos at 30°C. Blood islands formed earlier than normal in 21.9% of the embryos at 30°C, in 2.5% of those at 34°C, and in 10.2% of those at 40°C. The primitive streak was discontinuous or otherwise abnormal in 16.4% of the embryos incubated at 30°C compared to 3.4% of those at 40°C and none of those at 34°C. Holes or abnormal vesicles in the area pellucida were observed frequently in embryos

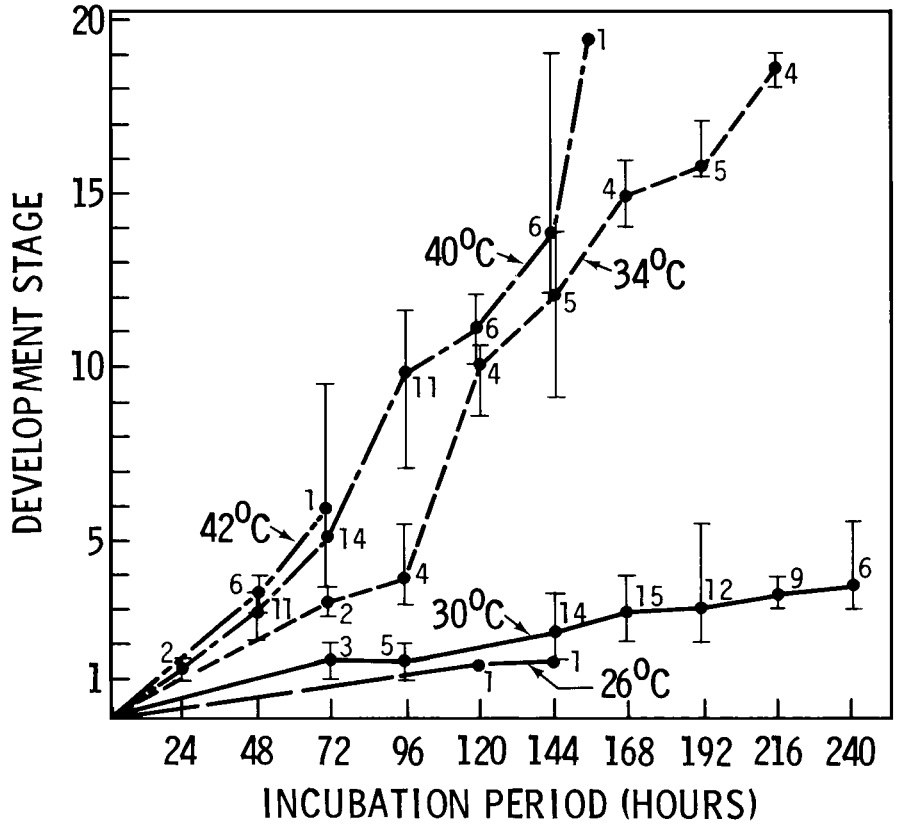

Fig. 2. Comparison of mean rate of development for eggs incubated at 26°, 30°, 34°, 40°, and 43°C. Numbers by points indicate sample size. Vertical lines through points on curves represent range for stage of development.

incubated at 30°C and 40°C (16.4% and 13.6% respectively) but these occurred in only 5.0% of those incubated at 34°C.

The observed frequency of occurrence of several of these abnormal conditions is dependent upon the stage of development attained. For example, abnormal brain development may have been observed in only one embryo incubated at 30°C merely

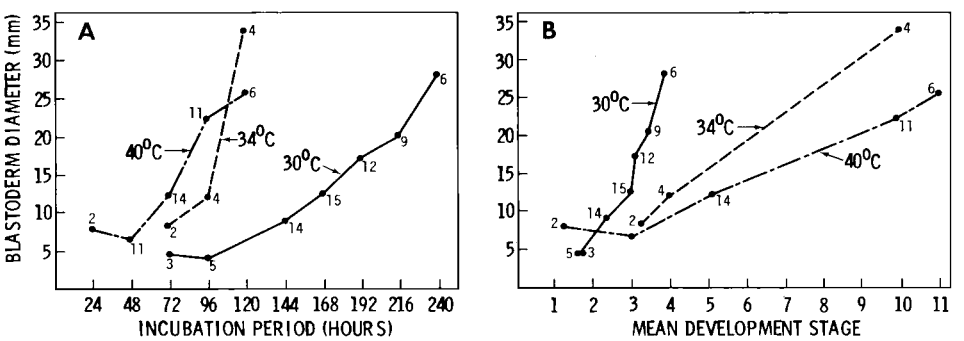

Fig. 3. Diameter of blastoderm relative to **A.** incubation period, and **B.** stage of development, for embryos incubated at three different temperatures. Numbers by points indicate sample sizes.

TABLE 1. Embryonic mortality and abnormal development with incubation at different temperatures for various incubation periods

Incubation temperature (C°)	Hours incubation	Sample size	Percent mortality	Percent abnormality
42	48	6	0	67
	72	5	80	60
	96	7	100	43
	129	5	100	0
	132	2	100	0
40	24	7	0	29
	48	13	0	46
	72	18	22	72
	96	17	24	88
	120	15	47	100
	144	9	33	89
	156	1	0	0
	168	8	100	75
34	72	6	0	0
	96	8	0	38
	120	6	0	67
	144	6	0	50
	168	4	0	0
	192	6	0	0
	216	4	0	0
30	72	3	0	67
	96	10	10	10
	144	15	7	33
	168	16	6	56
	192	12	0	75
	216	10	10	90
	240	7	14	86
26	120	4	25	0
	144	3	33	0
	168	4	0	0

because most embryos incubated at that temperature did not develop past the late primitive streak stage (stage 3 to 4). Also, a larger number of abnormalities can be defined for more advanced embryos that have more structures.

Incubation temperature also affected the differential growth rate. With equal periods of incubation the blastoderm was generally larger in embryos incubated at 40°C than in those incubated at 34° or 30°C (Fig. 3A). However, relative to stage of development, the blastoderm was larger in embryos incubated at 30°C than in those at 34° or 40°C (Fig. 3B). Figure 3A suggests that the absolute size of the blastoderm is directly determined by both the incubation period and the incubation temperature, but Figure 3B indicates that growth of the blastoderm relative to development of the embryo is inversely related to the temperature of incubation. The low temperature (30°C) inhibited embryonic development more than growth of the blastoderm (vegetative growth), and the high temperature (40°C) stimulated embryonic development more than vegetative growth.

DISCUSSION

Eklund and Charlton (1959) and Derksen (1975) reported mean incubation temperatures of 33.7° and 35.2°C respectively for naturally-incubated Adélie Penguin eggs. Because their methods precluded retention of live embryos in the eggs, these temperatures do not account for any rise in temperature due to increased metabolism

of the developing embryo (Romijn and Lokhorst 1956). These temperatures may approximate mean natural incubation temperatures during early embryonic stages when the embryo generates little metabolic heat. To eliminate the effects of Adélie Penguin embryo heat production, which may become increasingly significant after about 11 days of incubation, our studies dealt with incubation periods less than 11 days.

Due to variation caused by certain environmental, physiological, and behavioral factors affecting incubating penguins, the natural mean incubation temperature may not be the temperature at which development proceeds at the fastest rate with the minimum mortality and abnormal development (the optimum). The faster rate of development observed in this study with incubation at 34° (±1°C) compared to Herbert's (1967) results with incubation at 33.5°–34.0°C suggests that the optimum incubation temperature for Adélie Penguin embryos may be slightly higher than the accepted natural incubation temperature of 33.7°C (Eklund and Charlton 1959), at least for the early part of the incubation period. This difference could also be due to the different handling of eggs used in Herbert's (1967) study than in the present study. It is also possible that Adélie Penguin embryos at Cape Hallett have an inherently faster development rate than do those at Signy Island. Both Herbert's and our "optimum" incubation temperatures are within the mean egg temperatures reported by Huggins (1941) and others, and this shows that, in general, the thermal requirement for successful incubation (White and Kinney 1974) for Adélie Penguin embryo development is similar to that of other birds.

Since the primitive streak stage is only attained after 4–5 days of incubation, and the stages before that were hard to recognize, we did not open sufficient unincubated eggs to determine the incidence of held eggs. We assumed that this was not a significant factor for this study since there is a slow rate of development during the first 4 days of incubation, and it seemed unlikely that the penguin would hold an egg more than 24 h.

Adélie Penguin embryonic development rate increased with increasing incubation at temperatures from 26°–42°C (Fig. 2). This has been reported by numerous authors for most animals (e.g. Lillie and Knowlton 1898, Romanoff 1960, Patten 1958, Prince et al. 1969). These investigators also acknowledge a general increase in embryonic mortality and abnormal development as the incubation temperature approaches an upper or lower limit beyond which growth and development do not occur.

Adélie Penguin embryonic growth and development were severely impeded with incubation at 26°C but not entirely prevented. The temperature below which the embryo would not begin to develop is apparently below 26°C for this species, which seems reasonable because the domestic chicken physiological zero has been reported to be as low as 20.5°C (Edwards 1902).

The incubation temperature above which chick embryo growth and development do not occur has been reported to be 43° or 44–45°C (Dareste 1891, and Dumas 1924, respectively, as cited in Romanoff 1960). The upper temperature extreme for the Adélie Penguin embryo is somewhat higher than 42°C (±1°C), because six embryos incubated at that average temperature for 48 h were alive when examined. Embryos were unable to withstand this temperature for more than 2 to 3 days, however, for 14 embryos incubated for 4 days or longer were dead when examined.

The total observed mortality was 30% for 88 penguin embryos incubated at 40°C for up to 7 days but only 7% for 73 embryos incubated at 30°C for up to 10 days.

Prince et al. (1969) reported nearly equal overall mortality for mallard (*Anas platyrhynchos*) eggs incubated at high and low temperatures differing equally from the optimum. Dareste (1891, as cited in Romanoff 1960) suggested the same to be true for the chicken. Hamilton (1952a), however, stated that chick embryo mortality was greater, degree for degree, with incubation at temperatures above rather than below the optimum, and he cited corroborative data of Romanoff et al. (1938).

Retarded development at 30°C was most pronounced for somites, for no somites were visible in any penguin embryo incubated at this temperature. Romanoff (1960) reported that somite formation in the chick embryo was inhibited at temperatures below 33.5°C. Blood island formation is less impeded than somite formation at 30°C because blood islands were observed in nearly half of the embryos incubated for 8–10 days at this temperature.

Adélie Penguin eggs incubated at 40°C had a large number of abnormal embryonic conditions accompanying the increased rate of growth and development. Blastoderms without embryos occurred quite frequently in these eggs. Dareste (1891, as cited in Romanoff 1960) reported the occurrence of this abnormal condition in chicken eggs, but he found these abnormal blastoderms at low temperatures as frequently as at high temperatures. Only one such blastoderm was recognized at 26° or 30°C in this study, but it may be that incubation had not proceeded long enough for more of them to be discernible. Alsop (1919) reported that 90% of the chicken embryos developing at 39.4°C or above had abnormal nervous systems. The penguin embryos incubated at 40°C did not exhibit this type of abnormal development to such an extent, but many of these embryos had been under incubation for only short periods of time. Alsop (1919) also reported that this type of abnormality was common at low temperatures for the chicken, but this was not observed for the penguin embryo. In most embryos incubated at 26° and 30°C development was so inhibited that this type of abnormality was not recognizable even if present.

Similar effects of incubation temperature on the size of the area opaca (Fig. 3A) have been reported by other authors (Romanoff 1960, Edwards 1902) for the chicken embryo.

Our results agree generally with those reported by Prince et al. (1969) for the mallard, and Romanoff (1960), Edwards (1902), and others for the chicken. Further studies could more accurately determine the optimum incubation temperature, the absolute upper and lower temperature limits, and the effects of fluctuating temperatures on the Adélie Penguin embryo. The effects of humidity on the Adélie Penguin embryo have not been examined. (Prince et al. [1969] found less effect of humidity than of temperature for mallard embryos.) To establish these points would require the collection of large numbers of eggs, and one must consider whether this knowledge would be worth the risk of further decreasing penguin chick production since the decline in breeding penguin population from 63,000 pairs in 1959–60 (Reid 1964) to 48,000 pairs in 1970 (Fredrickson pers. comm.) may be partly associated with scientific studies at Cape Hallett.

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